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D_5 DOPAMINE RECEPTOR REGULATION OF REACTIVE OXYGEN SPECIES PRODUCTION AND BLOOD PRESSURE IN MICE

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D_1-like receptors have novel antioxidant properties in rat renal vascular smooth muscles, but the D_1-like receptor (D_1 or/or D_5) involved is not known. The D_5 receptor inhibits phospholipase D (PLD) activity in CHO and HEK-293 cells, expressing the human (h)D_5 receptor (hD_5R). Because phosphatidic acid, a product of PLD, activates NADPH oxidase, we hypothesized that the hD_5R inhibits NADPH oxidase activity, and thus, the production of reactive oxygen species (ROS). These effects may be important in the hypertension that develops in D_5R deficient mice. Systolic blood pressures (SBP, mm Hg), measured from the femoral artery under pentobarbital anesthesia, are higher in D_5R deficient ($D_5-/-$) (SBP=136±4, n=70) than in wild type mice ($D_5+/+$) (SBP=103±1, n=67, $P<0.05$). Tempol (10 mg/kg IV), a superoxide dismutase mimetic, acutely decreases blood pressure in pentobarbital-anesthetized $D_5-/-$ but not in $D_5+/+$ mice ($D_5-/-$, SBP=before, 134±2, after, 120±5, n=5; $D_5+/+$, SBP=before, 103±1, after, 100±3, n=9, $p<0.05$). The expressions of $p47^{phox}$, one cytosolic, and $gp91^{phox}$, one membrane component of NADPH oxidase, are increased in $D_5-/-$ mice kidney compared with $D_5+/+$ mice ($P<0.05$, n=3/group). In HEK-293 cells expressing hD_5R(HEK-hD_5R) but not D_1R, the D_1/D_5 agonist, fenoldopam (FEN), decreases NADPH oxidase activity, in a time- and dose-dependent manner (FEN, 5 μM, 30 min = 50±2 light units/μg protein; vehicle= 36±1, $P<0.05$, n=3/group). FEN also inhibits O_2^- by 58% (FEN, 5 μM = 42±5 nmol O_2^- /10^6 cells; vehicle= 101±4, $P<0.05$, n=8/group), and H_2O_2 production by 36% (FEN, 1 μM = 28±1.4 nmol H_2O_2/hour/10^6 cells; vehicle= 18±2.2 , $P<0.05$, n=4/group). cAMP/PKA does not mediate the inhibitory effects of the D_5R on ROS production; the adenylyl cyclase inhibitor, SQ22536, and PKA inhibitors, H-89, R-8-Piperidino-cAMPs and Rp-cAMPs, do not prevent the D_5R action. The D_5R also cofractionates with gp^{91phox} and p^{67phox} , in HEK-hD_5R and rat renal proximal tubule cells (RPTC). In RPTCs, the D_5R but not D_1R is linked to and co-fractionates with $gp91^{phox}$. We suggest that the D_5R inhibits NADPH oxidase activity, directly or indirectly, via PLD, but independent of PKA, decreases ROS production, and may explain the antihypertensive function of D_5Rs.

Key Words: D5 Receptor, Reactive Oxygen Species, Hypertension

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THE ROLE OF RENAL SUPEROXIDE DISMUTASE (SOD) ON THE SLOW PRESSOR EFFECTS OF ANGIOTENSIN II

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Low doses of Ang II increased BP and oxidative stress after 14 days in rats. However, Ang II downregulated renal extracellular Cu-Zn-SOD (ECSOD), a major antioxidant defense system, without alteration of Mn-SOD or intracellular SOD (IC-SOD). We tested the hypothesis that in the absence of EC-SOD, Ang II causes even greater oxidative stress. We used EC-SOD wild type (WT) and knockout (KO) mice, treated with a slow pressor dose of Ang II (400 ng/kg/min) or vehicle for 2 weeks. Oxidative stress was apparent in the KO mice, since the excretion of 8-PGF2α was higher in KO (WT: 1.3±0.1 KO: 2.2±0.3 pg/day, $p<0.01$), however Ang II increased 8-isoPGF2α only in WT (WT: 2.2±0.2, $p<0.01$;KO: 2.0±0.2,ns). Further, renal cortex superoxide (O2-) concentration was higher in KO mice and also was increased by Ang II only in WT (WT:7.8±0.4 KO: 11.8±1.2mmol/mg prot,$p<0.01$; WT+AngII: 12.5±1.4, $p<0.05$; KO+Ang II: 12.9±1.3mmol/mg prot). Excretion of malondialdehyde (MDA) was also higher in KO and was increased by Ang II only in WT (WT: 32±3; KO: 60±8 nmol/day, $p<0.01$; WT+Ang II: 60±5 KO+Ang II: 54±7, ns). Na excretion, GFR, RBF and FF were not different between WT and KO and Ang II had no effect on each. Ang II increased conscious MAP similarly in both groups (WT +15±3 vs KO +16±3 mmHg, ns). Under anesthesia MAP was also similar (WT Ang II: 98±3 vs KO Ang II: 102±3 mmHg, ns). Renal mRNA expression for Mn- and IC-SOD did not differ between WT and KO. In WT mice the expression of EC-SOD was lower in Ang II treated mice (−1.6 fold). However, after Ang II, expression of IC-SOD mRNA was higher in KO (WT: +0.9 vs KO: +1.9 fold, $p<0.01$) but Mn-SOD was not different. In conclusion, EC-SOD KO mice have greater renal oxidative stress. However, Ang II failed to generate further oxidative stress or higher MAP in the absence of EC-SOD in knockout mice, possibly due to upregulation of renal IC-SOD, which may defend against the increased production of superoxide.

Key Words: Oxidative Stress, Angiotensin II, Superoxide Dismutase

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RENAL ENDOTHELIN RECEPTOR TYPE B UPREGULATION IN RATS WITH LOW OR HIGH RENIN HYPERTENSION

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Endothelin-1 (ET-1) is a potent renal and systemic vasoactive peptide. It acts through ETA and ETB receptors. We investigated density and subtype distribution of ET-1 receptors in hearts and kidneys of normotensive and hypertensive rats.

Five groups of uninephrectomized Wistar rats were put on a low salt diet for six weeks. During this period, three groups of rats drank tap water and two groups received saline. One group of each regimen received DOCA subcutaneously (1.6 mg/day). The fifth group of rats had the left renal artery clipped to induce 1K1C hypertension. At 6 weeks, mean arterial pressure (MAP) was recorded in conscious rats via a femoral artery catheter. Binding assays using 125I-ET-1 were carried out on membrane preparations in the presence and absence of the ETA receptor antagonist FR139317.

On tap water, MAP was at 121.8±3.3 mmHg and DOCA or saline did not raise this MAP. On DOCA-salt and in 1K1C rats, MAP was increased to 154.5±5.8 mmHg ($p<0.001$) and 218.4±10.5 ($p<0.001$) mmHg, respectively. ET receptor subtypes were not equally expressed in the heart and the kidney: ETA was predominantly expressed in the heart, whereas ETB was predominant in the kidney. Both hypertensive models, the DOCA-salt and the 1K1C rats showed further significant changes: i) Cardiac weight index compared to controls of 2.49±0.06 mg/g was higher ($p<0.001$) at 3.89±0.10 and 4.86±0.18 mg/g in DOCA-salt and 1K1C hypertension, respectively, and kidney weight index compared to controls of 4.78±0.22 mg/g was higher at 10.10±0.54 mg/g in DOCA-salt ($p<0.001$) but tended to be below controls in 1K1C rats. ii) In the kidneys, the density of the ETB receptor subtype was upregulated in DOCA-salt and 1K1C rats from 160±8 to 217±12 and 190±2 fmol/mg ($p<0.05$), respectively, and ETA tended to be downregulated. iii) Plasma renin activity was decrased in DOCA-salt rats from 17±3 to 0.17±0.01 ng/ml/h and increased in 1K1C rats on low salt diet to 30±5 ng/ml/h ($p<0.001$).

We conclude that upregulation of the ETB receptor mediating vasodilation and downregulation of the ETA receptor mediating vasoconstriction is compatible with a mainly renal counterregulatory effect of

endothelin-1 to hypertension. This counterregulation may occur in both low and high renin models of hypertension.

Key Words: Endothelin Receptors, Hypertension, High or Low Renin

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LEFT VENTRICULAR HYPERTROPHY IN CHRONIC KIDNEY DISEASE

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Chronic kidney disease (CKD) is defined as the presence of kidney damage or decreased renal function for at least three months, and is associated with high prevalence of cardiovascular complications. While left ventricular hypertrophy (LVH) is the single strongest predictor of cardiovascular morbidity and mortality in dialysis population, very few studies are available on LVH prevalence and predictors in CKD.

Thus, we investigated 274 pts (183 men and 91 women, aging 17-78 yrs) referred to our outpatient clinic for CKD, during a three years period. All patients had blood pressure (BP) assessed by 24 hrs ABPM, left ventricular mass index (LVMi) measured by M-Mode echocardiography, creatinine clearance (CrCl) determined by Cockroft formula, hemoglobin (Hb), biochemical profile, and daily urinary protein excretion (U_{PROT}) by routine methods.

The values for 24 hr systolic (S), diastolic (D) BP and pulse pressure (PP) were 146±16, 88±11 and 56±14 mmHg respectively. The prevalence of arterial hypertension (BP≥140/90 mmHg) was 46%. Non dipping prevalence was 68%. CrCl was 47±34ml/min, Hb 11.6± 2.4 g/dl and proteinuria ranged from 0 to 7.6 g/day. LVMi was 156±49 g/m^2 bsa, and the prevalence of LVH (LVMi>125 g/m^2 bsa) was 71%. A direct relationship was demonstrated between LVMi and PP (p=.0006) and age (p=.008) respectively , while LVMi was inversely related to CrCl (p<.02) and Hb (p<.04). By stepwise regression analysis study, male gender (beta 31.12), Cr Cl (beta −.25) and PP (beta 1.04) resulted as significant predictors of LVH (p<.00001), and this model accounted for 28% of LVMi variance.When we considered the 191 pts with 3 to 5 stages CKD (Cr Cl< 60 ml/min), male gender (beta 28.85), Cr Cl (beta −.51) and PP (beta 1.21) were again the predictors of LVH (p< .00001), while age (beta 1.06), male gender (beta 39.82), daytime systolic BP (beta .63) and U_{PROT} (beta −4.10) predicted LVH (p< .00001) in the 83 subjects with normal renal function (CKD stages 1 and 2).

In conclusion the prevalence of LVH in CKD is much higher than previously reported. Together with unmodifiable factors like age and gender, BP load is the most important factor associated with LVH, while the role of anemia seems to be less important. Moreover LVH is associated with urinary protein excretion, a strong predictor of cardiovascular disease in hypertensive patients, and LVMi progressively increases as renal function worsens.

Key Words: Chronic Kidney Disease, Left Ventricular Hypertrophy, Arterial Hypertension

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URIC ACID, NEPHRON NUMBER AND THE PATHOGENESIS OF ESSENTIAL HYPERTENSION

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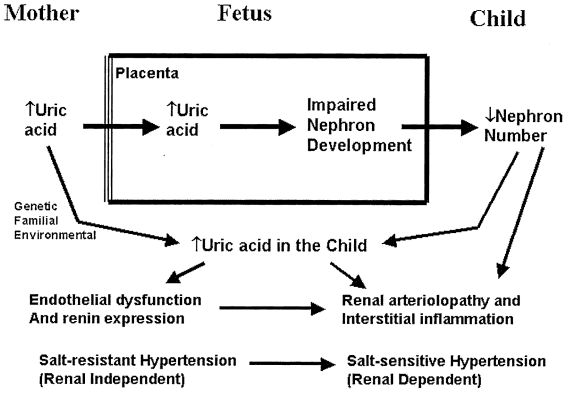
Genetic, physiologic and epidemiological studies provide clues but no clear elucidation of the etiology of essential hypertension. We have combined epidemiological methods, clinical trials, animal models and tissue culture investigations to demonstrate that uric acid may be a unifying link between several disparate theories of the origins of essential hypertension. In retrospective chart review of 265 newly diagnosed children with hypertension, we evaluated parental history, subject birth weight and serum uric acid. In an open label cross over trial we treated children with confirmed essential hypertension with allopurinol as a single anti-hypertensive agent. In tissue culture experiments we evaluated the effect of uric acid on glomerular endothelial proliferation by ^{3}H-thymidine uptake. Elevation of serum uric acid is closely associated with pediatric onset essential hypertension (r=0.80, p=0.00001). Maternal history of hypertension was preferentially associated with essential but not secondary or white coat hypertension (41.3% vs. 26.9% and 27.3%, respectively, p=0.009), as was lower birth weight (mean of 3070g vs. 3498g and 3512g, respectively, p=0.005). Uric acid (5mg/dl) added to the growth medium of glomerular endothelial in culture cells inhibited ^{3}H-thymidine uptake by 23% (p=0.03). These data, combined with our recent animal model evidence of uric acid inducing renal arteriolopathy and hypertension (through downregulatioin of eNOS and activation of the renin angiotensis system), the association of essential hypertension with congenitally lower nephron number, and the increased risk of hypertension in the children of preeclamptic mothers suggest that maternal uric acid may inhibit fetal nephron development. We propose the following model for the involvement of uric acid in decreased nephron number and childhood onset essential hypertension.



Key Words: Essential Hypertension, Etiology, Uric Acid

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INTENSIVE BLOOD PRESSURE CONTROL WITH VALSARTAN DECREASES PROGRESSION OF URINARY ALBUMIN EXCRETION IN NORMOTENSIVE TYPE 2 DIABETIC PATIENTS

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Diabetic nephropathy is the most common cause of renal failure in the US and is associated with cardiovascular morbidity and mortality. Multiple studies have demonstrated the benefits of blood pressure (BP) therapy on diabetic nephropathy but have focused on hypertensive diabetic patients with either micro- or overt albuminuria. There is a paucity of data regarding the effects of aggressive BP treatment in normotensive type 2 diabetic patients. The current study was designed to evaluate the effects of aggressive BP control with valsartan in a normotensive type 2 diabetic population with normoalbuminuria and microalbuminuria.

The study was a single-center, prospective, randomized clinical trial based in Denver, Colorado evaluating the effects of intensive versus moderate BP control on type 2 diabetic patients. We randomized 129 type 2 diabetic patients with a BP of <140/80-90 mm Hg without overt